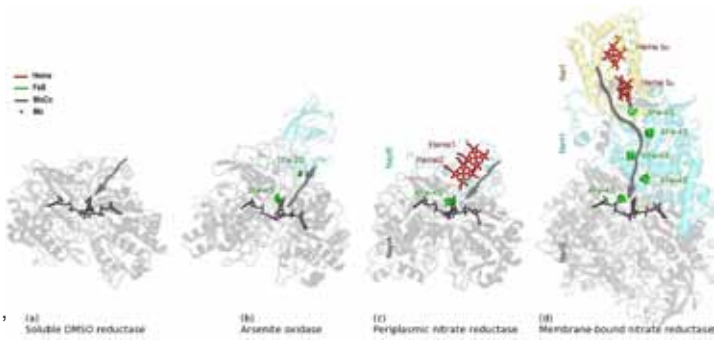
ERMOE: engineering the reactivity of complex molybdoenzymes. PCV 2006-2010

Molybdoenzymes of the DMSO reductase family have similar active sites, differ in quaternary structures and cofactor contents and specifically catalyse various oxo-transfer reactions. What determines specificity and directionality is unknown.

Methods, goals

A multidisciplinary approach:
Biochemistry, molecular biology, crystallography, bioinformatics, advanced EPR and electrochemical kinetics.
20 permanent researchers, biologists, physicists, chemists, in three research units:
• Laboratoire de Bioénergétique et Ingénierie des Protéines, CNRS UPR 9036 Marseille.
• Laboratoire de Chimie Bactérienne, CNRS UPR 9043, Marseille
• Laboratoire de Bioénergétique Cellulaire, CNRS/CEA UMR 6191, Cadarache.
Comparative studies of three enzymes: Arsenite oxidase, periplasmic and membrane bound nitrate reductases
Our goal was to study all aspects related to the function of these enzymes to elucidate the mechanism and determinants of substrate specificity.



Main results

1) **The interactions with membrane quinones and phospholipids [1-5]** Definition of the site of mena and ubi-semiquinones in membrane bound nitrate reductase, and activation of the enzyme by cardiolipin, using advanced EPR techniques.
2) **The electron transfer chain [6-8]** Definition of the redox, electronic and magnetic properties of the cofactors involved in long range electron transfer:
3) **The molybdenum active site [12-17]**. Using site-directed mutagenesis, identification of the aminoacids involved in substrate access and binding at the Mo. Combining EPR and electrochemical measurements, we discovered several inactive forms of the Molybdenum active site that have been isolated before and mistaken for catalytic intermediates, **paving the way for future research.**
4) **Evolutionary and physiological aspects of arsenic utilization [9-11]** Biochemical and phylogenetic investigations show that arsenite oxidase already existed in the anaerobic Archean to detoxify and produce energy. Arsenate reductase appeared later and was initially used to oxidize arsenite.

Outcome: 17 publications / 4 years, each involving 2 or 3 partners

1. Biogenesis of a respiratory complex is orchestrated by a single accessory protein. **J Biol Chem** 2007
2. High-stability semiquinone intermediate in nitrate reductase A (NarGHI) from Escherichia coli is located in a quinol oxidation site close to heme bD." **Biochemistry**. 2007
3. Direct evidence for nitrogen ligation to the high-stability semiquinone intermediate in Escherichia coli nitrate reductase A**.** **J.Biol Chem**. 2010
4. HYSCORE Evidence That Endogenous Mena- and Ubisemiquinone Bind at the Same Q Site (QD) of Escherichia coli Nitrate Reductase A" **J. Am. Chem. Soc.** 2010
5. Cardiolipin-based respiratory complex activation in Bacteria, **Proc. Natl. Acad. Sc. USA** 2011
6. New method for the spin quantitation of [4Fe-4S](+) clusters with S = 3/2. Application to the FS0 center of the NarGHI nitrate reductase from Escherichia coli. **J Phys Chem B** 2007
7. Arsenite oxidase from *Ralstonia :* characterization of the enzyme and its interaction with soluble cytochromes, **J Biol Chem**, 2010.
8. The Rieske protein: a case study on the pitfalls of multiple sequence alignments and phylogenetic reconstruction. **Mol Biol Evol** 2006
9. The small subunit AroB of arsenite oxidase: lessons on the [2Fe-2S]-Rieske protein superfamily**,** **J Biol Chem** 2010.
10. Enzyme phylogenies as markers for the oxidation state of the environment: the case of respiratory arsenate reductase and related enzymes**,** **BMC Evol Biol** 2008
11. Comment on "Arsenic (III) Fuels Anoxygenic Photosynthesis in Hot Spring Biofilms from Mono Lake, California" **Science** 2009
12. Access to the active site of periplasmic nitrate reductase: insights from site-directed mutagenesis and zinc inhibition studies. **Biochemistry** 2007
13. Correcting for electrocatalyst desorption and inactivation in chronoamperometry experiments. **Anal Chem**. 2009
14. Effects of slow substrate binding and release in redox enzymes: theory and application to periplasmic nitrate reductase**.** **J Phys Chem B** 2007
15. Dependence of catalytic activity on driving force in solution assays and protein film voltammetry: insights from the comparison of nitrate reductase mutants **Biochemistry** 2010
16. Major Mo(V) EPR signature of Rhodobacter sphaeroides periplasmic nitrate reductase arising from a dead-end species that activates upon reduction. Relation to other molybdoenzymes from the DMSO reductase family." **J Phys Chem B** 2008
17. Reassessing the strategies for trapping catalytic intermediates during nitrate reductase turnover" **J. Phys Chem B** 2010

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